学校编码：10384　　分类号_____密级_____
学号：20720080150003　　UDC_____

厦门大学

博 士 学 位 论 文

翡翠贻贝足丝粘附蛋白 Pvfp-1 粘附功能及其应用的研究

Adhesion and application research on green mussel (*Perna viridis*) adhesive foot protein 1 (Pvfp-1)

蒋 臻

指导教师姓名：张其清　教授/博导
孙亚楠　讲师
专 业 名 称：高分子化学与物理
论文提交日期：2011 年　月
论文答辩时间：2011 年　月
学位授予日期：2011 年　月

答辩委员会主席：_____
评　　阅　　人：_____

2011 年　月

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下,独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果，均在文中以适当方式明确标明，并符合法律规范和《厦门大学研究生学术活动规范（试行）》。

另外,该学位论文为(　　)课题(组)的研究成果，获得（　　）课题（组）经费或实验室的资助，在（　　）实验室完成。(请在以上括号内填写课题或课题组负责人或实验室名称，未有此项声明内容的，可以不作特别声明。)

声明人（签名）：

年　　月　　日

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

（　　）1.经厦门大学保密委员会审查核定的保密学位论文，于　　年　　月　　日解密，解密后适用上述授权。

（　　）2.不保密，适用上述授权。

（请在以上相应括号内打“√”或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

年　　月　　日

摘 要

海洋贻贝粘附蛋白（marine mussel adhesive proteins, MAPs）具有极强的粘附性、韧性和防水性，这使得贻贝粘附蛋白不仅在海水设备的防水、抗菌和密封等水下作业方面有着较广泛的应用前景，其良好的生物相容性更使其在生物医学领域（如粘接断裂骨骼、粘合软组织、改善材料表面生物活性等）也有重要的潜在应用价值。由于贻贝足蛋白Mfp-1（mussel foot protein 1）具有的粘附性能被认为是源自其含有DOPA的十肽重复序列，而翡翠贻贝足蛋白Pvfp-1（*Perna viridis* foot protein 1）的十肽重复序列并不含有DOPA。因此，本文根据Pvfp-1的结构特点，应用基因工程方法获得Pvfp-1及其几种缺失突变体。在分析对比这些缺失突变体的粘附功能并进而探讨其粘附机理的基础上，设计合成了同样源于Pvfp-1序列的多肽，通过分析对比其粘附性能来探讨粘附机理。同时，还研究了源自Pvfp-1的蛋白质和多肽作为生物粘合剂用于改善生物惰性材料PTFE表面生物活性的能力。主要研究内容和结论简述如下：

从新鲜翡翠贻贝足部提取总RNA并经PCR扩增后得到*Pvfp-1*基因编码区W-1236，以及缺失突变体R-900，R-240和C-237四种目的基因片段。将目的基因构建到表达载体中进行原核表达，并最终优化表达得到其中的两种缺失突变体蛋白R-240（Pvfp-1 N端8个十肽重复序列）和C-237（Pvfp-1 C端非重复序列）。通过亲和层析，纯化得到重组蛋白R-240和C-237。

通过粘附性能的对比分析发现，R-240和C-237都具有优于商业化Cell-Tak^{TM}的粘附性能。其中C-237经酪氨酸酶修饰后粘附能力更佳，而且在生物惰性材料PTFE表面具有较好的粘附及包被能力。经氨基酸序列、蛋白质二级结构以及表面形貌分析显示，C-237优异的粘附性能可能源于其序列中的DOPA含量和胶原类似结构域，以及所形成的致密空间网状结构。因此，Pvfp-1中的非重复区对其粘附性能起了重要的增强作用。细胞实验证实，C-237能促进细胞在玻璃尤其是PTFE表面的粘附以及铺展，而且没有细胞毒性。因此，C-237可以作为生物粘合剂用于改善生物惰性材料PTFE表面的生物活性，使之更好的应用于生物医学领域。

为了进一步研究清楚C-237优异的粘附性能，本研究设计合成了相应的一系列多肽。通过对比分析发现了其中粘附能力最佳的多肽C2(M)（源自Pvfp-1非重复区序列C-237，含有修饰后的DOPA）。经氨基酸序列以及表面形貌的对比分析发现，影响这些多肽粘附性能的机理在于氨基酸序列中DOPA的含量和位置，多肽的疏水性，以及表面致密的空间网状结构。这为设计开发其他多肽类生物粘合剂提供了理论依据。经细胞实验证实，在生物惰性材料PTFE表面具有极佳粘附及包被能力的多肽C2(M)能显著促进细胞在PTFE表面的粘附以及铺展，而且没有细胞毒性。因此，多肽C2(M)也可以作为生物粘合剂用于改善生物惰性材料PTFE表面的生物活性，使之更好的应用于生物医学领域。

关键词：Pvfp-1；生物粘合剂；生物惰性材料

Abstract

Marine mussel adhesive proteins (MAPs) have properties such as strong adhesion, intensity, and moisture-resistance. These characteristics make MAPs attracted increasing attention for its potential application in underwater sealants and adhesives. Since MAPs are biocompatibale and biodegradable, they have considerable importance in biomedical engineering such as being applied as bioadhesive. Mfp-1 (mussel foot protein 1) was considered as the key protein for coating and adhesion because it contains repeating decapeptides and DOPA. However, decapeptides in Pvfp-1 (*Perna viridis* foot protein 1) contains no DOPA. Therefore, we used genetic engineering to obtain Pvfp-1 and several of its deletion mutants for determining their coating and adhesion ability. According to the results, several peptides were designed for studing their adhesion mechanisms. Furthermore, we also studied the potential abilities of recombinant protein and peptide that originated from Pvfp-1 to be used as bioadhesive which could improve bioactivity of bio-inert surface. The main contents and results are as follows:

Total RNA was extracted from the foot tissue of *Perna viridis* and the whole coding region of *Pvfp-1* gene (W-1236) and several of its deletion mutants (R-900, R-240, C-237) were amplified. These fragments were constructed into expression vector for prokaryotic expression. After induced by IPTG under the optimized condition, two of these recombinant proteins were successfully expressed. They are R-240（eight repeating decapeptides of N-terminal of *Pvfp-1*）and C-237（non-repeating region of C-terminal of *Pvfp-1*）. Affinity chromatography was used for purifying these recombinant proteins for they contain His-Tag.

Using adhesion analysis, we found that both of R-240 and C-237 displayed comparable adhesion ability to that of Cell-Tak^{TM}, espetially C-237 after DOPA modification. And modified C-237 also showed outstanding coating and adhesion ability on the non-adhesive PTFE surface. We analyzed the amino acid sequence, protein structure and surface morphology of these two proteins, and found out that the

excellent adhesion ability of C-237 might be due to the DOPA content and collagen domain in its amino acid sequence, and its three dimensional network structure. Therefore, the non-repeating region of Pvfp-1 has made important contribution to its adhesion ability. Moreover, C-237 showed better cell adhesion and spreading ability on both glass and PTFE surfaces and was non-toxic. Therefore, recombinant C-237 could be used as bioadhesive for medical purpose and be potentially used as an improver for bio-inert materials when applied in biomedical areas.

A series of peptides according to the sequence of C-237 and Pvfp-1 were designed. Using adhesion analysis, we found that peptide C2(M) (derived from the non-repeating region of Pvfp-1, contains modified DOPA) displayed superior coating and adhesion ability, especially on bio-inert surface of PTFE. We also analyzed the amino acid sequence and surface morphology of these peptides, and found that the factors which could influence adhesion might be the DOPA content and position in peptide, the hydrophobicity of peptides, and the three dimensional network structure of peptide. These findings provide suggestions for designing other peptide-based adhesives. Moreover, after coating with peptide C2 (M), cell adhesion and spreading on the bio-inert PTFE was improved and the peptide coating was non-toxic to cells. Therefore, peptide C2 (M) is suitable for converting bio-inert PTFE surface into bioactive one, and shows the possibility to be used as a bioadhesive on other bio-inert materials when applied in biomedical areas.

Key words: Pvfp-1；bioadhesive；bio-inert material

目 录

Contents